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A Comparison of Methods for the Analysis of Hydrocarbons in Marine Sediments

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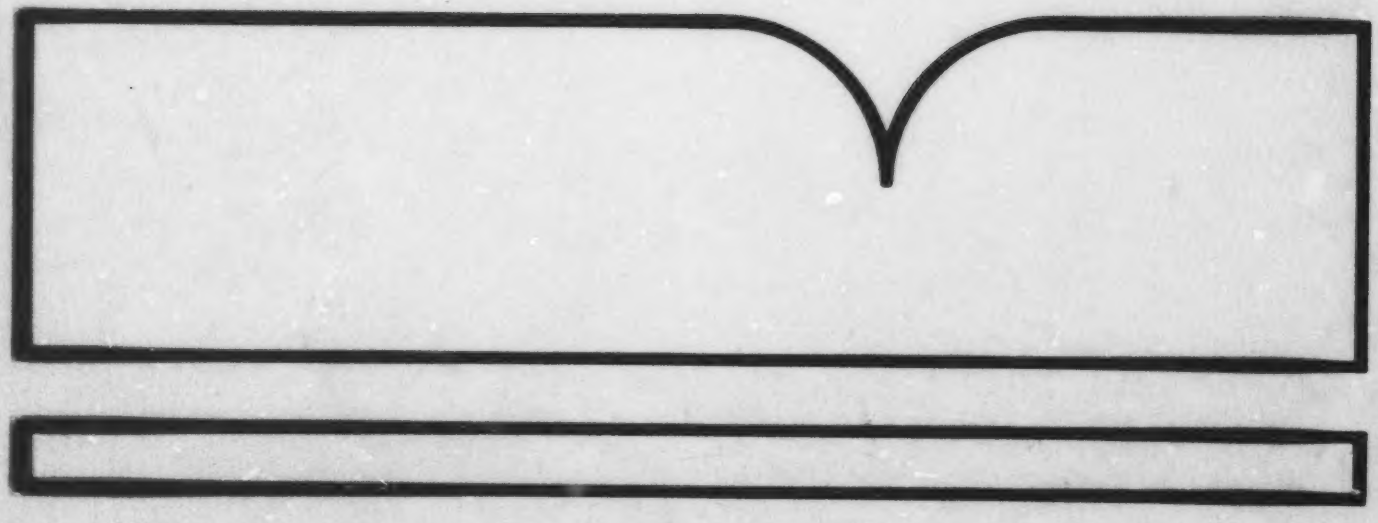

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16. ABSTRACT

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A Comparison of Methods for the Analysis of Hydrocarbons in Marine Sediments

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The ability of several methods to extract polycyclic aromatic hydrocarbons (PAHs) and other petroleum hydrocarbons from marine sediments was examined. Comparisons of soxhlet and methylene chloride reflux methods gave extraction efficiencies that showed no statistical difference in the return of PAHs; however, the return when using ball-mill tumbling was significantly lower. The relative content of individual parent PAH compounds, parent compound distributions (PCDs), and alkyl homologue distributions (AHDs) of PAHs was calculated using capillary column GC–MS. The similarities of the distributions showed that any of the three methods could have been used to calculate these distributions. An examination of several extraction methods commonly used for the extraction of petroleum hydrocarbon material from sediment samples showed differences in results that depended on the extraction method employed. In addition, the methods varied in their ability to extract resolved versus unresolved material and to return aliphatic and aromatic hydrocarbon compounds.

Analytical determinations of the quantities and types of pollutant hydrocarbons entering the marine environment are essential for an understanding of the fate and effects of these compounds in marine systems. Since substantial amounts of these hydrocarbon compounds are deposited in marine sediments, research studies have examined the content of polycyclic aromatic hydrocarbons (PAHs) and other petroleum hydrocarbons in marine sediment samples.

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Several studies have examined the total PAH content, the relative content of individual parent PAH compounds, that is, parent compound distributions (PCDs), and the alkyl homologue distributions (AHDs) of PAHs from extracts of marine sediments (*1–9*). The analytical methods used in these studies were generally complex and lengthy, and consisted of an extraction, an isolation of the PAH material (by either complex formation or chromatographic separation), and an analysis. Based on comparisons of PCDs and AHDs of the sediment samples with those of samples from known origins (e.g., petroleum, combustion products), these studies discussed the origins of PAH compounds found in sediments.

Researchers have utilized numerous techniques to determine the content and composition of petroleum hydrocarbons in marine sediments. Unfortunately, no standard technique for the analysis of hydrocarbons from sediments exists, and the variety of techniques utilized has made the comparison of results from different studies nearly impossible. Recent publications have examined extraction and analytical methods (*10, 11, 12*), while other studies have intercalibrated on a standard reference sediment (*13*). In a study using different solvents for extraction, it was concluded that benzene was more efficient than hexane or chloroform in extracting hydrocarbon material from sediment (*10*). It was found, however, that the percentages of each class of hydrocarbons (alkane, cyclic alkanes, and aromatics) extracted by the solvents were relatively similar. The efficiency of soxhlet extraction, followed by alkaline hydrolysis, was compared with that of acid wash prior to soxhlet extraction (*11*). For the methods examined, no substantial differences in extraction efficiencies were found. An interlaboratory comparison of trace level (ppb) hydrocarbons present in two marine sediment samples was discussed (*13*). Eight procedures using different extraction methods and different methods of analysis resulted in considerable differences in estimates of the concentrations of hydrocarbons and in determinations of the most abundant aliphatic and aromatic compounds present. While the latter study showed that considerable variation among laboratories resulted from different extraction and analytical methodologies, other factors inherent to the sediment samples (e.g., total organic carbon, geophysical parameters, acute vs. chronic contamination) may also affect extraction efficiency.

This chapter reports the results of experimentation designed to examine the extraction and characterization of petroleum hydrocarbons, including PAHs, from marine sediments. The efficiencies of several methods for the extraction of PAHs from marine sediments were compared, and the ability of these extraction methods [followed by gas chromatographic–mass spectrometric (GC–MS) analysis] to reproduce consistently AHDs and PCDs from contaminated sediments was exam-

ined. The research involving petroleum-contaminated samples included a two-laboratory intercalibration, an experiment that compared the ability of several sediment extraction methods to recover different classes of petroleum hydrocarbons from sediments, and a study that examined the effectiveness of methods for extracting aliphatic and aromatic hydrocarbons from recently contaminated sediments.

Methods

The sediment samples used in the present study were obtained from Narragansett Bay. The sediment sample used for the PAH extraction study and in Experiments 1 and 4 was obtained adjacent to a pier in Narragansett, Rhode Island. The intercalibration sediment (ICS) was obtained near the north end of Jamestown Island (*14*). Recently contaminated sediments were produced by dispersing sediment obtained near the south end of Jamestown Island in seawater, dosing it with No. 2 fuel oil in a continuous flow oil-dosing system (*15*), and collecting it after it had settled to the bottom of the tanks.

To ensure uniformity, sediments were thoroughly mixed before use. The ICS was wet-sieved through a 1.0-mm sieve, followed by resuspension in distilled water and mixing on a ball mill for 2 hr. The intercalibration sediment was then filtered to remove most of the water. Other sediments were thoroughly mixed by hand. Replicate analysis of aliquots of these sediments showed subsampling variability was small (Table II).

Brief descriptions of the three methods used for extracting PAH material from sediment samples are listed in Methods section (Methods I–III). Method II was adapted from a method used by another laboratory (*16*). Aliquots of the extracts obtained from these methods were analyzed by splitless, injection-glass-capillary-column GC–MS techniques. Concentrations of PAH compounds were calculated by integrating the extracted ion current profiles (EICPs) corresponding to the molecular ions of the compounds of interest. Examples of EICPs for the $Z = -22$ homologue series are shown in Figure 1. Z numbers are determined from the molecular formula C_nH_{2n+Z}. Specific peaks in the EICPs were not integrated if their spectra did not correspond to those of the compounds of interest or if they did not have characteristic retention times. The integrated values were normalized and displayed in a semilogarithmic format as PCDs and AHDs. PCDs represent the relative concentrations of the parent compounds of interest, that is, PAHs with molecular weights of 178, 202, 228, 252, 276, and 278. These distributions were obtained by correcting the raw data for instrument response with the aid of response factors calculated from known PAH standards. In the few cases where a standard was not available, raw data were corrected by the use of another

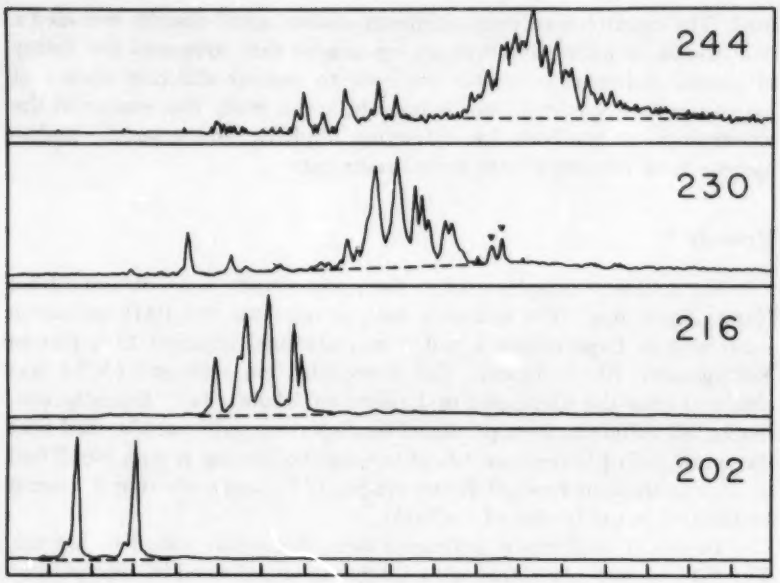

Figure 1. Extracted ion current profiles (EICPs) of the molecular ions for the Z = −22 AHD for a marine sediment. Arrowheads indicate the $(M + 2)^+$ ions of compounds with MW = 228.

PAH standard with the same molecular weight. AHDs show the concentration of the parent compound in relation to its C_1–C_3 alkyl homologues. AHDs were not corrected for instrument response because of a lack of necessary alkylated standards.

Both PCDs and AHDs are helpful in graphically demonstrating the similarities and/or differences among samples. Figure 2 shows the total ion current profiles obtained from samples of coal tar from a dock and sediment from the Charles River, Boston. The numbers above the peaks refer to tentative identifications listed in Table I. The differences in the PAH content of the samples are graphically demonstrated by the shapes of the PCDs and by the lengths of the Z = −22 AHDs (Figure 3). The sediment exhibits a much larger proportion of higher-molecular-weight PAHs. Similar distributions have been found in other samples, and these characteristic distributions have been used to provide evidence as to the origins of PAH compounds in marine environments (*8*).

The methods used for the extraction of petroleum hydrocarbon compounds are listed in Methods section (Methods III–VII). In an attempt to demonstrate the efficiency of Method III relative to Method V,

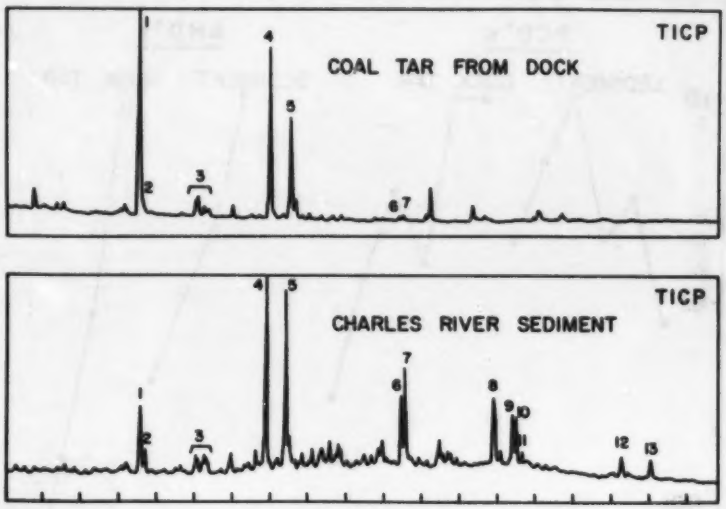

Figure 2. A comparison of total ion current profiles (TICPs) of a dock tar and sediment. Numbers refer to compound identifications presented in Table I.

Table I. PAH Compounds in Figure 2

Compound Number	*Molecular Weight*	*Z Number*	*Tentative Compound Identifications*
1	178	−18	phenanthrene
2	178	−18	anthracene
3	192	−18	C_1-phenanthrenes + C_1-anthracenes
4	202	−22	fluoranthene
5	202	−22	pyrene
6	228	−24	benzanthracene
7	228	−24	chrysene
8	252	−28	benzofluoranthene
9	252	−28	benzo[*e*]pyrene
10	252	−28	benzo[*a*]pyrene
11	252	−28	perylene
12	276	−32	6-ring PAH
13	276	−32	6-ring PAH

successive sample extractions were applied to some sediments and the resulting extracts were analyzed. Results were compared to determine whether any extractable hydrocarbons remained following the initial extraction.

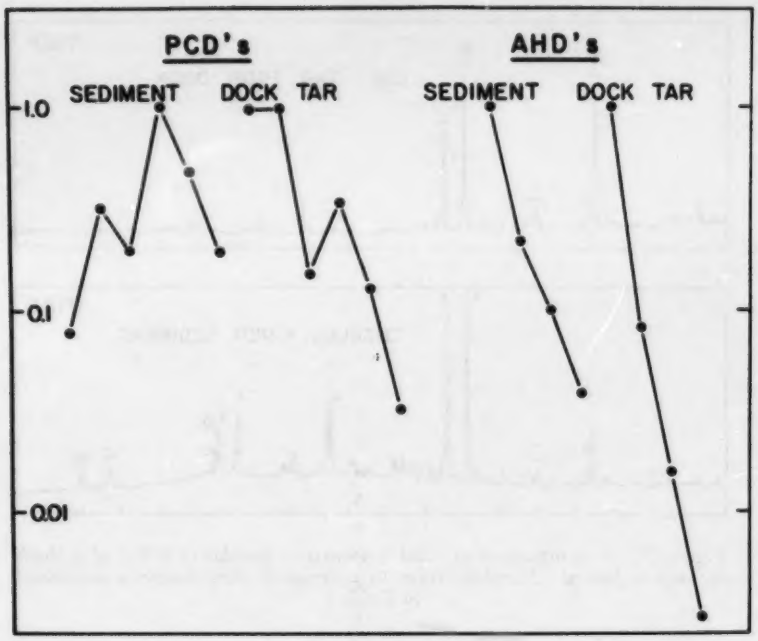

Figure 3. A comparison of PAH PCDs and Z = −22 AHDs of a sediment and a dock tar

The organic fractions resulting from all extractions were purified by passage through a column of silica gel, sodium sulfate, and copper powder. Extracts from Methods II, III, IV, and VI were reduced in volume on a Kuderna–Danish apparatus, solvent-exchanged with hexane, and reduced in volume to 1 mL under a stream of nitrogen. The extracts from Methods I, V, and VII were handled similarly but were evaporated to dryness (to remove benzene, toluene, and methanol) before being redissolved in hexane.

The sample extract was then separated on a 45-cm × 1-cm-o.d. column of silica gel (100–200 mesh, deactivated with 5% water) into F-1 (aliphatic) and F-2 (aromatic) fractions. Final analysis was accomplished by gas chromatography (GC) or GC–MS.

The GC analyses were performed on several glass capillary columns with various liquid phases (OV-101, SE-52, and SE-54) in a Hewlett-Packard 5840-A GC with splitless injection. Except for the intercalibration study, quantitation of chromatograms was based upon external standards. Areas from chromatograms were manually integrated using a digitizing planimeter.

The GC–MS analyses were performed on similar glass capillary columns in a Shimazdu Model GC-4CM GC connected to a Finnegan 1015 mass spectrometer equipped with a Systems Industries data system with Riber 400 D-8 software. The mass spectrometer was operated in the EI (electron impact) mode at 70 eV.

Reagent blanks and standard samples were periodically processed to ensure continued satisfactory performance of the methods.

Methods for the Extraction of Hydrocarbons from Sediment

Method I. (Soxhlet): Set up soxhlet with 10–200 g wet sediment in the extraction thimble, and 125 mL methanol and 125 mL benzene in the attached boiling flask. Reflux 24 hr. Stop with extraction chamber filled with solvent and exchange extract in boiling flask for 100 mL benzene. Reflux 24 hr. Combine extracts with 25 mL water and 25 mL pentane, shake vigorously, and separate aqueous and organic phases. Wash organic phase with 25 mL water. Extract combined aqueous phases with approximately 10 mL pentane. Combine organic phases.

Method II. (Ball-mill tumbler): Dewater 10–200 g wet sediment with a 2-fold excess of methanol (based upon the calculated dry weight). Repeat. Extract sample by rotating in a 3-fold excess of CH_2Cl_2 at 5 rpm for 18 hr. Repeat for 7 and 64 hr, respectively. Methanol and CH_2Cl_2 fractions are combined and filtered and then partitioned into aqueous and organic phases by adding an equal volume of water. Extract aqueous phase with 20 mL CH_2Cl_2 and combine organic phases. Repeat partitioning steps.

Method III. (Methylene chloride reflux): Weigh 10–200 g of wet sediment into a flask. Based upon the calculated dry weight, add a 3-fold excess of CH_2Cl_2 and a 1.5-fold excess of distilled water. Reflux mixture for 2 hr while stirring with a magnetic stirrer. Centrifuge. Separate organic and aqueous phases. Wash sediment twice with 25 mL CH_2Cl_2 and 25 mL H_2O. Separate aqueous and CH_2Cl_2 phases and combine all CH_2Cl_2 fractions.

Method IV. (Shaking with CH_2Cl_2): Weigh 25–50 g of wet sediment into a centrifuge bottle. Based upon the calculated dry weight, add a 2-fold excess of CH_2Cl_2 and extract the sediment by shaking for 5 min. Centrifuge. Draw off supernatant and repeat twice. Separate CH_2Cl_2 and aqueous phases in a separatory funnel. Retain and combine all CH_2Cl_2 phases.

Method V. (Methanolic saponification/benzene): Weigh 10–200 g of wet sediment into a flask. Based upon the calculated dry weight, add a 5-fold excess of 0.5*N* methanolic NaOH and a 2.5-fold excess of benzene. Add a volume of distilled water equal to 10%–20% of the volume of the

aqueous phase. Reflux mixture for 2 hr while stirring with a magnetic stirrer. Centrifuge. Separate organic and aqueous phases. Extract the aqueous phase with 50% of its volume of CH_2Cl_2. Wash sediment twice with approximately 25 mL CH_2Cl_2 and 25 mL H_2O. Combine each washing with the aqueous phase, extract by shaking, and separate the CH_2Cl_2. Combine all CH_2Cl_2 fractions.

Method VI. (Saponification): Weigh 10–200 g of wet sediment into a flask. Based upon the calculated dry weight, add a 4-fold excess of 0.5*N* NaOH. Reflux mixture for 2 hr while stirring with a magnetic stirrer. Centrifuge. Extract the aqueous phase with 50% of its volume of CH_2Cl_2. Wash sediment twice with approximately 25 mL CH_2Cl_2 and 25 mL water. Combine each washing with the aqueous phase, extract by shaking, and separate the CH_2Cl_2. Combine all CH_2Cl_2 fractions.

Method VII. (Methanolic saponification/toluene): Follow Method V, but substitute a 5-fold excess of a mixture consisting of 70% 0.5*N* methanolic KOH and 30% toluene, for the methanolic NaOH and benzene (*21*).

Results

PAH Investigation. The steps in the PAH procedures following extraction were examined by the addition of a standard containing alkylated and nonalkylated PAH compounds. A comparison of the quan-

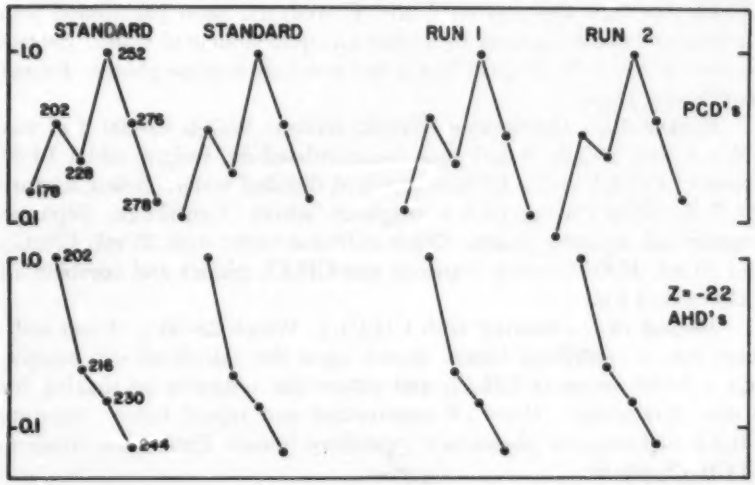

Figure 4. Comparison of the PCDs and the Z = −22 AHDs of a PAH standard before (standards) and after (Runs 1 and 2) being carried through the volume reduction and column chromatography portions of the sample preparation procedure

tity of nonalkylated PAH in the standard with the amount returned after it was carried through the volume reduction and chromatographic steps of the procedure showed that there was an average difference of 5%. Comparisons of the PCDs and AHDs from the standard and from the results of this experiment (Figure 4, Runs 1 and 2) showed that this portion of the procedure caused little alteration of these PAH distributions. Comparisons of phenanthrene/anthracene ratios obtained from the standard with those from Runs 1 and 2 showed no significant differences. These data indicate that no significant photooxidation of susceptible PAHs had occurred.

The results of the three PAH extraction methods are shown in Figure 5. The soxhlet extraction (I) and CH_2Cl_2 reflux (III) were about equal in their ability to extract PAH compounds. The ball-mill tumbler (II) was only 72% as efficient as the other methods. A two-way, crossed-factor analysis of variance on the log-transformed data was used to test whether or not there were significant differences in the amount of parent compounds returned by these three methods. An F-test indicated signifi-

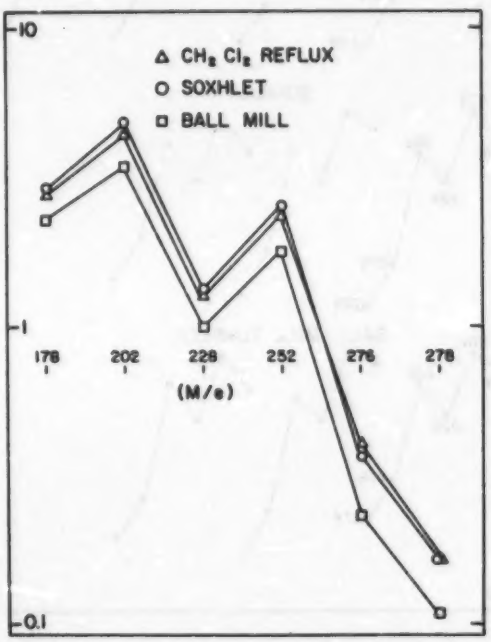

Figure 5. Recovery [log (ppm dry weight)] of nonalkylated PAH compounds for methylene chloride reflux, soxhlet, and ball-mill tumbler extraction methods

cant differences ($\alpha = 0.05$). The Student–Newman–Keuls test (*17*) showed that Method III and Method I returns were not significantly different from one another ($\alpha = 0.05$), while the return from Method II was significantly lower than the other methods ($\alpha = 0.05$). All PCDs and $Z = -22$ AHDs (Figures 6 and 7) from the three methods were similar, as were other AHDs at $Z = -18$, $Z = -24$, and $Z = -28$. Therefore, any of these three extraction methods could have been used to characterize the PAH compounds in this sediment sample.

The high resolution inherent in a capillary column analysis proved valuable when characterizing the PAH assemblages in the samples, but the low-temperature tolerance of the glass capillary column was found to preclude the collection of data on PAHs with molecular weights greater than 300.

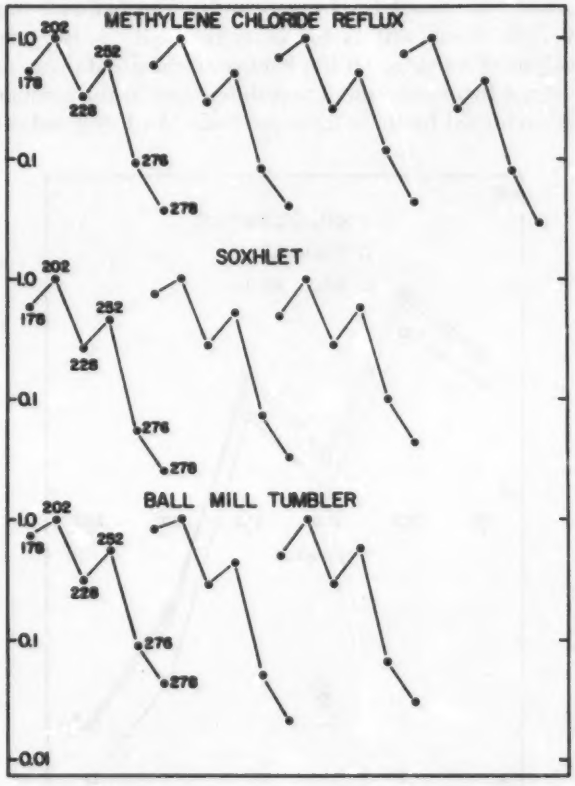

Figure 6. Comparison of PAH PCDs for replicate extractions by methylene chloride reflux, soxhlet, and ball-mill tumbler methods

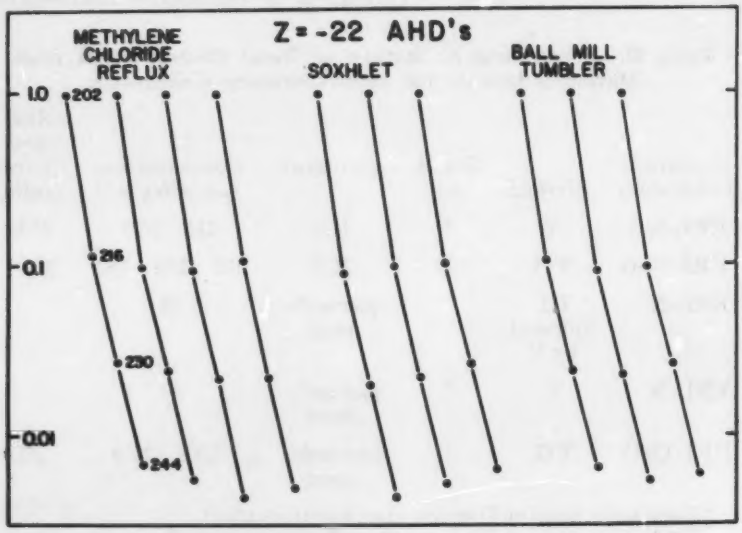

Figure 7. Comparison of PAH Z = −22 AHDs for methylene chloride reflux, soxhlet, and ball-mill tumbler extraction methods

The collection of full-mass-range spectral data is advantageuo because it allows the molecular ions of the compound of interest to b distinguished from fragment ions of other compounds, so that only th peaks of interest are included in the EICP area calculations. However this mode of data acquisition is not as sensitive as select ion monitoring.

Hydrocarbon Investigations. The results of Experiments 1–4 which compared the ability of five commonly used methods to extract petroleum hydrocarbons from sediments, are listed in Tables II–V.

Experiment 1 (Table II) was an intercalibration between this laboratory (EPA Environmental Research Laboratory—Narragansett) and the laboratory of James Quinn (University of Rhode Island, Graduate School of Oceanography) using aliquots of the ICS and the pier sediment samples. The amounts of hydrocarbon material extracted from both sediment samples were very close when similar extraction methods (V and VII) were used.

Experiment 2 (Table III) compared the extraction efficiencies of three methods for the recovery of hydrocarbon material from ICS samples. Method IV, the most rapid and least vigorous (shaking with CH_2Cl_2), was only about 17% as efficient as Method V (reflux and saponification with MeOH, NaOH, and benzene, followed by extraction with CH_2Cl_2). Method VI (NaOH reflux followed by CH_2Cl_2 extraction) was only about 31% as efficient as Method V.

Table II. Experiment 1: Return of Total Hydrocarbons from Marine Sediments for Interlaboratory Calibration

Analytical Laboratory	*Method*	*Standard*	*Sediment*	*Concentration (μg/g dry wt)*	*Average Concentration*
ERL-N	V	[a]	ICS	215 210	213
URI/GSO	VII	[a]	ICS	213 236 193	214
ERL-N	III followed by V	[b]	pier sediment	33	
ERL-N	V	[b]	pier sediment	33	
URI/GSO	VII	[a]	pier sediment	33.8 32.9	33.4

[a] Quantitation based on Docosane as an internal standard.
[b] Quantitation based on No. 2 fuel oil as an external standard.

Experiment 3 (Table IV) compared the abilities of Methods V and III (CH_2Cl_2 reflux) to extract sediments recently contaminated by No. 2 fuel oil. Method V (the more vigorous extraction) returned more hydrocarbon material from the sediment samples than did Method III. The difference in return, however, was primarily in the form of unresolved complex material (*11*) that was believed to have resulted from the long-term chronic contamination of these sediments while they were in Narragansett Bay. Both methods returned approximately equal amounts of resolved material.

Experiment 4 (Table V) examined sediments obtained near a pier in Narragansett Bay. Two extraction methods were used alone and in sequence to examine their ability to remove hydrocarbons from the sediment samples. The results of Method III followed by Method V on

Table III. Experiment 2: Comparison of Extraction Efficiencies of Methods IV, VI, and V for an ICS Sediment Sample[a]

Method	*F-1*	*F-2*	*Total*	*Average*
IV	38	2	40	36
	30	2	32	
VI	62	2	64	64
	63	2	65	
V	189	16	205	206
	197	11	208	

[a] Quantitation based on No. 2 fuel oil as an external standard.

Table IV. Experiment 3: Comparison of Extraction Efficiencies of Methods III and V for a Narragansett Bay Sediment Exposed to No. 2 Fuel Oil in an Oil-Dosing System[a]

	Concentration (μg/g dry weight)								
	F-1			*F-2*			*Total*		
Method	R	U	*Total*	R	U	*Total*	R	U	*Total*
III	38	111	149	9	37	46	47	148	195
	37	105	142	10	39	49	47	144	191
V	37	172	209	15	47	62	52	219	271
	36	161	197	12	47	59	48	208	256

[a] Quantitation based on No. 2 fuel oil as an external standard. R = Resolved peak areas on chromatograms. *U* = Unresolved peak areas on chromatograms.

Table V. Experiment 4: Comparison of Extraction Efficiencies of Methods III and V[a]

		Concentration (μ/g dry weight)		
Part[b]	*Method*	*F-1*	*F-2*	*Total*
A	III	4	19	23
	V	8	2	10
	Total	12	21	33
B	V	10	23	33

[a] Quantitation based on No. 2 fuel oil as an external standard.
[b] Part A: Sample extracted by Method III followed by Method V. Part B: Sample extracted only by Method V.

the same sample showed that more hydrocarbon material was returned by the more vigorous Method V. Most of this additional material, however, was returned as unresolved complex material and was found primarily within the F-1 (aliphatic) fraction. The second extraction with Method V returned approximately 200% additional F-1 material, but only 11% additional F-2 (aromatic) material. Method III therefore appeared to be adequate for extracting F-2 (aromatics) from these contaminated sediments but inefficient for the extraction of F-1 (aliphatic) material.

Reagent blanks processed periodically yielded no material that would have interfered with the analyses above.

Discussion

PAH Investigations. Studies examining the input of PAHs to marine systems have examined the PAH assemblages present in samples of petroleum (*6*), airborne particulates (*18, 19*), water particulates (*19*), combustion products (*18*), and coal tar (*8*). The AHDs and the PCDs

of PAHs found in marine sediment have been used to indicate the sources and origins of these compounds in marine benthic environments (*8*). A variety of extraction and analytical techniques has been utilized to characterize PAH assemblages in samples; however, many of the techniques involved complex and lengthy extraction and isolation procedures.

The present study compared the ability of three techniques to extract PAH compounds and their alkylated homologues from marine sediments. Specifically, this study was designed to determine if the results of a shorter and simpler extraction (CH_2Cl_2 reflux) were comparable with those from longer yet more commonly used extraction methods (exhaustive soxhlet and ball-mill tumbling).

Prior to conducting the extraction efficiency experiments, an examination of the volume reduction and column chromatographic portions of the procedure was undertaken to determine if selective photooxidation or other processes were altering the characteristic PCDs and AHDs. Previous studies examining PAH methodology have demonstrated that radiation from room light can destroy some PAH molecules (*20*). It was found that the light degraded anthracene, napthacene, benzanthracene, dibenz-[*a,c*]anthracene, pyrene, benzo[*a*]pyrene, benzo[*e*]pyrene, benzo[*ghi*]-perylene, and coronene on silica gel plates, but did not degrade phenanthrene, chrysene, and triphenylene. In the present study two aliquots of a PAH standard were taken through the latter portions of the procedure. The PCDs and $Z = -22$ AHDs of the standard are shown with those of the replicate runs in Figure 4. The quantities of nonalkylated PAH material returned from this portion of the procedure were only slightly different than those of the standard. The PCDs were very similar and the AHDs were nearly identical. In addition, calculation of the phenanthrene/anthracene ratios showed that the average ratio for the initial standard was not significantly different from that for Runs 1 and 2. These findings indicated that no significant losses or alterations of PAHs occurred during the volume reduction and column chromatographic portions of the procedure.

The results of the three extraction experiments for PAH compounds are shown in Figures 5, 6, and 7. The concentrations of nonalkylated parent PAH compounds at *m/e* 178, 202, 228, 252, 276, and 278 were determined by GC–MS analysis. These concentrations (corrected for instrument response) are shown in Figure 5. The results showed that the soxhlet extraction and the CH_2Cl_2 reflux were approximately equal in their ability to extract these PAH compounds, while the ball-mill tumbler extraction method was only about 72% as efficient as the other two methods. PCDs obtained from the different extraction methods are shown in Figure 6. This figure demonstrates the consistency of each method for extracting nonalkylated PAH compounds and shows the close similarity

of PCDs obtained from these different extraction methods. The AHDs were calculated for the Z = −22 homologue series from extracts obtained from the three extraction methods. The close agreement of the C_0 through C_3 AHDs is graphically demonstrated in Figure 7. Other AHDs at Z = −18, Z = −24, and Z = −28 calculated for these three extraction methods were also very similar.

A capillary column inlet system and collection of full-range mass spectral data offer several advantages over other inlet systems (e.g., packed column GC, direct probe) and/or limited-scan-range data acquisition modes (e.g., select ion monitoring). For example, with packed column, solid probe, or batch inlet systems, it would be exceedingly difficult to resolve certain isomers (e.g., phenanthrene and anthracene) whose relative abundances may prove valuable in evaluations of the origins, sources, and pathways of PAH compounds in the marine environment. In addition, the capillary GC inlet allows calculation of retention time data that can be used in conjunction with other data to include or delete peaks from EICP integrations (described in next paragraph). Unfortunately glass capillary columns have a limited temperature range. AHD data up to the C_3 homologue of MW = 252 compounds can be obtained, but column life is shortened by the high temperature needed to elute these compounds. As glass capillary column technology improves, the temperature limit may increase and thus allow the examination of higher-molecular-weight PAHs.

The collection of full-scan-range data from samples allows the spectra of peaks to be checked. From an examination of the spectra, peaks can be included or deleted from EICP area calculations. An example of this is shown in Figure 1, where EICPs for *m/e* 202, 216, 230, and 244 are presented. The C_2 homologue is quantified by integrating the area under the *m/e* 230 EICP. An examination of the retention times and the spectra of the major peaks in the EICP indicated that the two peaks to the right of the main group resulted from the M + 2 ions of the benzanthracene and chrysene compounds (MW = 228). These peaks, therefore, were not included in the calculation of the C_2 alkyl homologue. A disadvantage of collecting full-scan-range data from GC–MS is that of decreased sensitivity. The loss in sensitivity usually precluded the collection of data for AHDs above the C_3 alkyl homologue. While this is a distinct disadvantage, an examination of data collected by the present study and from other research shows that the shape of the AHD is usually defined by the first four points. Therefore, depending on the research, calculation of the alkyl homologues above C_3 may not be necessary.

Hydrocarbon Investigations. Since no standard method exists for the analysis of hydrocarbon material from marine sediment, the extraction and analytical procedures used to determine the level of hydrocarbons in

samples of marine sediments have varied substantially. This study compared the ability of five methods commonly used for petroleum hydrocarbon analysis (Methods section, Methods III through VII) to extract aliphatic and aromatic hydrocarbons from sediment samples.

The results from the intercalibration study (Experiment 1) between the EPA Environmental Research Laboratory—Narragansett and the laboratory of James Quinn at the U.R.I. Graduate School of Oceanography agreed closely (Table II). This agreement also indicates that subsampling variability of the intercalibration sediments was quite low. The amount of material recovered from sediments by three extraction methods (Table III) was compared in Experiment 2. A considerable increase in the amount of hydrocarbon material returned from sediment samples was observed as the method of extraction became more vigorous.

The results of Experiment 3 are shown in Table IV. This experiment compared the ability of different methods (III and V) to extract recently added petroleum hydrocarbons from marine sediment. The more vigorous extraction (Method V) increased the yield, but the additional material returned was unresolved. It appeared that the extractions were equally effective in returning the resolved hydrocarbons. In the unresolved portions of chromatograms, it was not possible to differentiate between recently added hydrocarbons and those from long-term inputs. This differentiation may be important for determining the extent of contamination from oil spills where extensive areas are impacted and control or prespill sediment samples (to determine background hydrocarbon levels) are not available.

In Experiment 4 the same sediment sample was extracted by Methods III and V successively. This experiment (Table V) showed that the saponification (Method V) returned additional material from the sediment; however, most of this material was unresolved and in the F-1 fraction. The amount of additional F-2 material returned by the second extraction was only about 11%. This experiment showed that a method may be suitable for one class of petroleum hydrocarbons but not be as effective for recovering other classes.

Conclusions

A comparison of three extraction methods used for recovery of PAH material from sediments showed that the CH_2Cl_2 reflux and the soxhlet extraction were approximately equal in their ability to extract PAH material; however, ball-mill tumbling was only approximately 72% as efficient. The similarities of the AHDs and PCDs obtained from the three extraction methods indicate that any of these methods could have been utilized to characterize the PAH assemblages in the sediment; but these findings

could not necessarily be generalized to include all PAH contamination levels and all sediment types. Full-mass-range data acquisition and a glass capillary GC inlet to the mass spectrometer were advantageous for the calculation of AHDs and PCDs, but these adaptations limited the sensitivity of the method and the range of compounds that could be examined.

An examination of several extraction methods commonly used for the extraction of petroleum hydrocarbon material from sediment samples showed differences in results depending upon the extraction method employed. In addition, the methods varied in their ability to extract resolved versus unresolved material and to return different classes of compounds (aliphatics and aromatics).

The factors considered in this paper and other variations in the extraction and analytical scheme (e.g., a method's ability to return lower-molecular-weight compounds, sediment-dependent differences in extractability, differences in analytical instrumentation) indicate that close agreement in results from laboratories utilizing different extraction methodologies should not be expected. While these variations may be acceptable to those who understand that the extraction of hydrocarbon material from a complex matrix (such as marine sediment) is still the subject of research studies, these variations may complicate the assessment of ecological damage resulting from inputs of hydrocarbon contaminants to the marine environment. These considerations, the results of the present study, the variation in results from other intercalibration studies (*13*), and the difficulties encountered when results from different studies are compared, suggest that a standard method of extraction and analysis of hydrocarbon material from sediments should be established. An increased amount of research effort should be directed toward this problem.

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